Iron Chelation With Desferrioxamine B in Adults With Asymptomatic Plasmodium falciparum Parasitemia

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To determine if iron chelation therapy has activity against human malaria, we administered desferrioxamine B in amounts of 100 mg/kg per day by continuous 72-hour subcutaneous infusions to 28 volunteers with asymptomatic Plasmodium falciparum infection in a randomized, double-blind, placebo-controlled crossover trial. Peripheral blood concentrations of P. falciparum ring forms were determined at 12-hour intervals in all subjects and serum concentrations of desferrioxamine B + ferrioxamine (the iron complex of desferrioxamine B) were measured in 26 subjects. Geometric mean concentrations of asexual intraerythrocytic parasites decreased with both chelator and placebo treatment, but the decrement with desferrioxamine B was significantly greater than that with placebo (P < .006) during both the initial and crossover periods. Compared with placebo, desferrioxamine B treatment was associated with an almost 10-fold enhancement of the rate of parasite clearance during both phases of the trial (P < .007). Mean ± SEM steady state concentrations of desferrioxamine B + ferrioxamine were 6.90 ± 0.60 µmol/L at 36 hours and 7.72 ± 0.68 µmol/L at 72 hours; in vitro, the ID₅₀ has been reported to be approximately 4 to 20 µmol/L. No drug toxicity was detected. Parasitemia recurred in 19 of 24 participants followed-up over 1 to 6 months. We conclude that desferrioxamine B enhances the clearance of P. falciparum parasitemia and that iron chelation may provide a new strategy to be developed for the treatment of malaria.

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Desferrioxamine B, a naturally occurring trihydroxamic acid derived from cultures of Streptomyces pilosus, is the only drug currently available for clinical use as an iron chelator. A generally safe and nontoxic agent, it must be administered by continuous parenteral infusion to be effective; daily doses of up to 150 mg/kg are used for therapy of iron overload. Preclinical data suggested that desferrioxamine B might have an antimarial effect in humans: (1) a mean plasma concentration of 20 µmol/L was achieved when desferrioxamine B was administered by continuous parenteral infusion to seven non-iron-loaded adults at 100 mg/kg/d; (2) the growth of P. falciparum in cultured erythrocytes was suppressed when desferrioxamine B in concentrations of 2 to 20 µmol/L was added to the medium, and (3) parenteral administration of desferrioxamine B in doses of 60 to 125 mg/kg/d inhibited parasitemia with P. vinckeii and P. berghei in rodents and P. falciparum in Aotus monkeys. Desferrioxamine B is apparently able to enter parasitized red cells, and the available evidence suggests that the chelator exerts its antiparasitic effect by binding intracellular parasite-associated iron and making it unavailable to essential plasmoidal enzymes. To determine if iron-chelation therapy with desferrioxamine B has activity against human infection with P. falciparum, we administered subcutaneous infusions of the drug to partially immune adults with asymptomatic parasitemia.

MATERIALS AND METHODS

Study design. The study was approved by the Ethical and Research Committee of the University of Zambia and by the Committee on Human Investigation of MetroHealth Medical Center, Case Western Reserve University. The study was conducted from February to August 1990, encompassing both months when malaria transmission is high and when it is low. Informed consent was obtained from all participants. Partially immune subjects with asymptomatic parasitemia were identified by obtaining thick blood smears from healthy, nonpregnant, and nonlactating community members, staining with Giemsa, and examining for the presence of P. falciparum ring forms. In this endemic area, subjects with asymptomatic parasitemia probably include (1) individuals with a low but steady level of parasitemia as a result of
long-standing immune clearance and (2) persons with a subclinical episodic infection. Study preparations (desferrioxamine B or placebo) were administered by continuous subcutaneous infusions via portable, battery-operated pumps (Cormed ML-6-4; Corning, NY) and Medfusion 300 (Medfusion Systems, Duluth, GA). Desferrioxamine B (Desferal) was donated by Ciba-Geigy, Ltd (Basel, Switzerland). Placebo solutions consisting of normal saline were prepared by the pharmacy of Macha Hospital.

Twenty-eight individuals were entered into a randomized, double-blind, crossover trial comparing desferrioxamine B (100 mg/kg/d) and placebo; subjects included 13 men and 15 women with ages ranging from 15 to 56 years, median 18 years. The participants received subcutaneous infusions for 6 days: desferrioxamine B and placebo were each administered for 72 hours with the sequence of administration determined by random assignment. At the end of each 72-hour period, any remaining solution was measured. Finger puncture blood samples were obtained at approximately 12-hour intervals to prepare thick blood films for the quantification of ring forms. Venous blood was collected in tubes containing potassium-EDTA at 72-hour intervals to determine blood counts. Serum samples were obtained at 36-hour intervals and frozen within 12 hours at −5°C to −10°C for later determinations of concentration for the first 72-hour period, but three did not continue for the second 72-hour infusion. Two of these volunteers declined further participation because of discomfort at the infusion site and inconvenience in wearing the pump. The third individual was removed from the study because she developed mild headache and low-grade fever that was initially presumed to represent clinical malaria. She immediately was treated empirically with chloroquine, but subsequently the parasite count was found to be 0 at the time of these symptoms and her illness was considered likely to have been of viral origin. An infusion pump malfunctioned on one occasion and the subject using the pump was changed to intravenous infusion of part of the placebo infusion and all of the desferrioxamine B solution. Another volunteer who was scheduled to use the defective pump was instead administered both desferrioxamine B and placebo intravenously. Among the 27 participants who received desferrioxamine B, the mean (±SEM) amount of the chelator that was actually infused over 72 hours was 290 ± 4 mg/kg, or 97% of the planned quantity of 300 mg/kg.

For the three participants who completed only the initial phase of the crossover trial, peripheral blood asexual parasites decreased from 600/μL to 0/μL and from 300/μL to 0/μL in the subjects who received desferrioxamine B, and from 185/μL to 110/μL in the individual who received placebo.

Geometric mean concentrations of peripheral blood parasites over two 72-hour treatment periods for the 25 individuals who completed the crossover trial are shown in Fig 1. At time 0, geometric mean peripheral blood parasite concentrations were similar in the 12 subjects who received desferrioxamine B first (1,148 rings/μL; SE range of 776 to 1,698) and the 13 subjects who were administered placebo first (1,319 rings/μL; SE range of 832 to 2,089) (P was not significant). Geometric mean concentrations of ring forms decreased throughout the crossover trial with both treatments. Decreases with placebo were significant during the initial phase (P = .002) and approached but did not reach significance during the crossover phase (P = .06), while declines with desferrioxamine were significant during both phases (P = .0001 for each period). Notably, when the magnitudes of the declines in mean parasite concentrations during desferrioxamine B therapy were compared with those with placebo, the decrements with desferrioxamine B were found to be significantly greater both in the initial (P = .005) and crossover (P = .0001) periods. The rates of parasite clearance during the crossover trial are shown in Fig 2 as the proportional decreases in parasite concentration over the two 72-hour periods of study, expressed logarithmically. Compared with placebo, desferrioxamine B treatment was associated with an almost 10-fold enhance-
ment of the rate of parasite clearance during both the initial 
(P = .006) (Fig 2A) and crossover (P = .0001) (Fig 2B) 
phases of the trial.

Ring forms were cleared from the peripheral blood after 
desferrioxamine B in 25 of 27 subjects. Rare gametocytes 
were observed in the peripheral blood smears of some 
volunteers; their numbers did not decline with desferriox-
amine B therapy. No toxicity was detected during the infusions 
of desferrioxamine B. Participants reported mild swelling 
and pain at the site of needle insertion during 22 of 25 
subcutaneous administrations of desferrioxamine B but 
only 10 of 25 subcutaneous administrations of placebo 
(P < .05).

Follow-up malaria smears. Repeat malaria smears were 
obtained from 24 of the 27 participants who received 
desferrioxamine B a median of 4 months after participating 
in the study (range, 1 to 6 months). Thick smears were 
positive in 19 (79%) of these individuals even though they 
were asymptomatic.

Serum concentrations of desferrioxamine B. Determina-
tions were performed on serum samples obtained at 36 
hours and 72 hours after beginning the infusion of desfer-
rioxamine B in 26 subjects. Mean (±SEM) steady state 
concentrations of desferrioxamine B + ferrioxamine were 
6.90 ± 0.60 μmol/L at 36 hours and 7.72 ± 0.62 μmol/L at 
72 hours; these means are not significantly different.

DISCUSSION

Our study was designed to determine if iron chelation is 
potentially useful in the treatment of human malaria. The 
emergence of resistance to all of the antimalarials currently 
in use provides the rationale for evaluating new therapeutic 
strategies. As a World Health Organization panel noted 
recently, “Most of the standard antimalarial drugs have 
been in use for 30 years or more and the increasing problem 
of drug resistance and the failure to reduce the transmis-
sion of malaria in many regions have emphasized the 
limitations of these drugs and made the search for new and 
more effective compounds imperative.”37 In the present 
study we attempted to reduce parasitemia in asymptomatic 
adults by exploiting the dependence of the malarial parasite 
on the essential nutrient, iron.

We used the only iron-chelating agent now approved for 
clinical use, desferrioxamine B, recognizing that this drug 
would be unlikely to find widespread clinical application 
because of its expense and the requirement for parenteral 
administration. When administered orally, desferrioxamine 
B is poorly absorbed.28 To be effective, the drug must be 
administered by continuous subcutaneous or intravenous 
infusion.29 In the dose and duration used in this study, 
desferrioxamine B could lead at most to the excretion of a 
few milligrams of iron in a person with normal or reduced 
iron stores,30 a quantity that would not substantially affect a 
patient’s iron content. Furthermore, a recent study suggests 
that malaria infection in humans is not influenced by iron 
status,31 and it has been shown that the antimalarial action
of desferrioxamine B in laboratory animals is independent of host iron status.\textsuperscript{22} We did not include a control period with the administration of ferroxamine (desferrioxamine B bound to iron) in our experimental design, but note that ferroxamine has been examined in Plasmodium falciparum grown in erythrocytes in vitro\textsuperscript{14} and in Plasmodium vivax infection in mice\textsuperscript{31}; no inhibition of the growth of parasites was observed.

To avoid withholding standard antimalarial therapy from patients admitted to hospital with clinical malaria, we chose to examine the effect of the chelator in asymptomatic adults found to have Plasmodium falciparum parasitemia in village surveys. These individuals continued to live and work in their communities while wearing portable infusion pumps for the duration of the study. For this initial trial, we chose 72 hours as the minimum period of treatment that might be expected to show activity of the drug. While anticipating that a single 72-hour course of desferrioxamine B would permit detection of an antimalarial effect, this duration of therapy was considered unlikely to result in the complete elimination of the parasite. Studies in vitro have provided evidence that desferrioxamine B is cytocidal against Plasmodium falciparum, but suggested that the chelator acts specifically at the late trophozoite stage of the intraerythrocytic parasite.\textsuperscript{10,32} The intraerythrocytic life cycle of Plasmodium falciparum lasts for about 48 hours,\textsuperscript{30} but is not well synchronized in contrast to other species of malaria; the life cycle in ‘laggards’ may last considerably longer.\textsuperscript{28} A chelator dose of 100 mg/kg/d produces serum concentrations in the range of the reported ID\textsubscript{50} for Plasmodium falciparum in vitro, but might not consistently achieve levels that lead to 100% inhibition of the parasite. Thus, a single 72-hour subcutaneous infusion of desferrioxamine B (100 mg/kg/d) would be unlikely to expose all parasites to lethal concentrations of the chelator at the susceptible stage of parasite development.

Given the restrictions of our experimental design, the results of the double-blind, placebo-controlled trial of desferrioxamine B provide unequivocal evidence that this iron-chelating agent has antimalarial activity against human infection. As shown graphically in Figs 1 and 2, geometric mean concentrations of Plasmodium falciparum asexual forms decreased throughout the crossover trial with both treatments. The overall mean decline during placebo periods presumably resulted from immune clearance of the parasites. With desferrioxamine B, the decrement in geometric mean parasite concentrations was almost 10-fold greater than that found with placebo therapy during both the initial and crossover periods. Thus, the iron-chelating agent seemed to exert a potent antimalarial effect, with complete clearance of Plasmodium falciparum parasitemia in more than 90% of those treated. The steady state mean serum concentrations (7 to 8 \(\mu\)mol/L) of desferrioxamine B achieved with predominantly subcutaneous administration of the drug in doses of 100 mg/kg/d in this study were at the lower end of the inhibitory range of 2 to 20 \(\mu\)mol/L and the range of values reported for the ID\textsubscript{50} 4 to 20 \(\mu\)mol/L, as determined in vitro.\textsuperscript{15,39,40} The reappearance of ring forms of Plasmodium falciparum in the peripheral blood of 24 of 27 of the subjects within 1 to 6 months of participating in the study could represent recrudescence or reinfection.

In general, desferrioxamine B is a safe drug. However, visual disturbances have been reported even with short-term desferrioxamine B therapy in non-iron-loaded patients,\textsuperscript{33,34} suppression of T-cell activation is a possibility with iron chelation therapy,\textsuperscript{17} and there appears to be an increased risk for certain infections with the long-term use of desferrioxamine B.\textsuperscript{19} While we did not detect any of these complications, we again point out that formal ophthalmologic and otologic evaluations were not possible in our study.

In summary, these results affirm that Plasmodium falciparum parasitemia is reduced in humans during iron chelation therapy. This finding suggests that iron chelation is a potential new chemotherapeutic strategy in malaria and that orally active iron chelators under development might find application as antimalarials.\textsuperscript{35} Oral chelators under study at the present time with documented antimalarial activity in vitro include pyridoxal isonicotinoyl hydrazone,\textsuperscript{4} the \(\alpha\)-ketohydroxypyridines,\textsuperscript{4} phenolic ethylenediamine derivatives,\textsuperscript{3} and desferriothiocin.\textsuperscript{2} An important task for the future would be to study the impact of iron chelation therapy on severe malaria in the nonimmune host and the seriously ill semi-immune patient. Although it must be administered parenterally, desferrioxamine B itself has potential application in the treatment of severe forms of malaria if iron chelation will be found to be effective in that setting.\textsuperscript{36}

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REFERENCES


Iron chelation with desferrioxamine B in adults with asymptomatic Plasmodium falciparum parasitemia [see comments]
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